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REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

Claims 1, 2, 8, 10, 16, 17, 41, 42, 45 are currently amended and claims 7, 12-15, 18-29, 31-40, 44 and 46-57 are canceled as being directed to non-elected subject matter. Applicant reserve the right to file one or more divisional applications on the subject matter of these claims. New claims 58-63 are added. Claims 1-6,8-11, 16, 17, 30, 41, 42, 43, 45 and 58-63 are presently pending.

As the claim to the specific antibody made by hybridoma, DSM ACC 2241 is considered free of the art, it is requested that full examination of additional species and the generic claims continue.

Applicants confirm the election of Groups I and II and species A, the antibody produced by the hybridoma DSM ACC 2241. Applicants have not canceled claim 30 as it is directed to a method of using the elected antibody. Further, applicants point out that rejoinder of process of using the product claims commensurate in scope with the product claims found allowable is permitted under the *Ochiai* guidelines. Applicants request rejoinder of claim 30 upon allowance of the product claims.

This amendment changes claims in this application. The amendments to the claims have support in the specification as filed. Specifically, the amendments to claims 1 and 17 are supported for the insertion of "human" on page 1 and throughout the specification and for "one or more surface markers' on page 6, lines 11-16, Figure 8 and Table 1. Amendments to claims 6, 16 and 17 directed to antibody fragments or derivatives are supported on page 9, line 26 to page 11, line 21, and particularly on page 10, lines 9-11, where antibodies or antibody fragments that bind to the DCs are described.

New claims 58 and 59 are supported with Example 11, beginning on page 66. New claims 60 and 61 are supported by Example 12, beginning on page 68 which shows two other species of antibodies that recognize the same antigen that is recognized by the M-DC8 antibody, designated as DSM ACC2241. New claims 61 and 62 are supported in Example 8

on page 63 and in Figures 9 and 10 that discloses the variable heavy and light chain regions of M-DC8 antibody. Amendments to claim 41 is supported on page 44 of the specification.

Information Disclosure Statement

Applicants note that the Examiner crossed off certain cited patent documents and publications in the PTO-1449. Applicants herewith provide an English abstract of EP 0 404097, an enlarged version of the Hsu publication and wish to point out that the Galy publication was from a book entitled Dendritic Cells: Biology and Clinical Applications, Chapter 1, from Academic Press (1999), which is clearly legible at the bottom of the pages of the document. It is requested that the Examiner kindly consider the abstract and enlarged copy at this time.

Objections to the Specification

The Examiner has objected to not the specification for not containing references to all priority data. Applicants have amended the specification to contain this information and it is requested that this rejection be withdrawn.

Further, the Examiner has objected to the presence of embedded hyperlinks and/or other forms of browser-executable codes. Applicants have delete these hyperlinks from the specification

The Examiner has objected to the Abstract as being too long and applicants have attached a new abstract on a separate sheet of paper that complies with the length requirement of an abstract.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 1-6, 8-11 and 16-17 are rejected as being indefinite and to this end, the Examiner has cited specific language in the referenced claims that he finds to be unclear and ambiguous as follows.

A) Claims 1 and 17

Claims 1 and 17 are rejected for the recitation of the phrases "reacts with an epitope," "DCs displaying features of" and "does not react with" as unscientific terms. Applicants have amended claims 1 and 17 to recite, as suggested by the Examiner, "binds" instead of "reacts." Additionally, "displaying features...." has been amended to recite "displaying surface marker antigens..." This language is supported on page 6, lines 11-16. As supported by pages from Immunology, 6th Ed., Roitt *et al.* (2001) pages 15 and 27, persons skilled in the art knew that the "CD system" was a system derived from analysis of mAbs against human leukocyte antigens. Applicants submit that these clarifying amendments overcome the Examiner's rejections of these claims.

B) Claim 2

Claim 2 is rejected as allegedly vague and indefinite for the recitation of "wherein the DCs represent a DC population of a maturational stage between immature and mature DCs." Claim 2 has been amended to recite" comprise" rather than "represent" to make it clear that the recited DCs are composed of a population of human DCs that overlaps with two major subsets of DCs; i.e., immature and mature DCs, that have previously been defined by several investigators and described in the pertinent literature (see references 2, 3 and 20 cited in the specification.) Furthermore, Figure 8 of the present application provides a diagram showing the overlap between these two subsets of DCs, immature and mature. Applicants submit that it is clear to the skilled person that "maturational stage" between immature and mature DCs is not indefinite in view of the specification. It is requested that this rejection be withdrawn.

C) Claim 5

Claim 5 is rejection as vague and indefinite for the recitation of "DCs of a restricted size and granularity." Applicants point out that a person skilled in the art, especially a molecular biologist or a physician trained in hematology knows how size and granularity of blood cells, particularly of lymphocytes and monocytes, can be determined. See for example, Immunology 6th Ed., Roitt *et al.*, pages 168-169, which shows in time-lapse video how the identification and movement of granules in T cells can be tracked. Additionally, Rick, a text

book in German that shows granules in lymphocytes, monocytes and plasma cell (and not considered by the Examiner in the IDS because the document is in German) shows how size and granularity of blood cells can be determined. Even thought Rick is not in English, page 48 shows representations of different cells showing different types of granularity. Thus, applicants submit that a person skilled in the art would not find the claims to be indefinite.

D) Claims 8 and 16

Claims 8 and 16 are rejected as indefinite for the recitation of "...DCs are recognized by the antibody." Applicants have amended these claims to recite "binding" and believe that this language should obviate this rejection.

E) Claim 10

Claim 10 is rejected as vague and indefinite for the recitation of "a continuous cell line." Applicants have amended claim 10 to delete "continuous" and believe that this amendment overcome this rejection.

F) Preamble to the Claims

The Examiner has requested that "Claims" prior to the start of the claims be changed to "WE CLAIM" and this amendment has been made.

In view of all of the amendments, arguments and supporting documentation that has been provided, applicants request that all of the above rejections based on indefiniteness be withdrawn.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 8, 9 and 11 are rejected as containing subject matter which was not described in the specification is such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims 8, 9 and 11 all recite specific hybridoma cell lines identified by DSM numbers. To overcome this rejection, the Applicant hereby submits evidence of the deposit with the DSM – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmgH. A

copy of the International Form from the DSM evidencing the deposit of the hybridoma ACC2241 is hereby attached. Further, the declaration by the undersigned attorney avers that the replacement and availability of the hybridoma as requested by the Examiner.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 6, 8-9 and 16-17 are rejected as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention.

Claims 6, 16 and 17 are rejected as containing language to "an antibody fragment" or "a derivative thereof" which the Examiner alleges encompasses a potentially unlimited genus when no genuses are disclosed.

Applicants have amended claims 6, 16 and 17 to specifically recite that the fragments or derivatives of the antibody are capable of binding to the epitope (claims 6 amd 17) or to the DCs (claim 16). Applicants submit that the antibody and the hybridoma that produces the antibody are provided, and thus fragments of such antibodies can be prepared and tested for the capability of binding to the same epitope as the complete antibody binds. In view these clarifying amendments, it is requested that this rejection be withdrawn.

Rejections under 35 U.S.C. § 102(b)

Claims 1, 6, 10 and 16-17 are rejected as being clearly anticipated by WO 93/04187 (of record). The Examiner alleges that WO 93/04187 teaches a monoclonal antibody which reacts with DCs and not other PBMCs, a cell line and a method of preparing an antibody. However, applicants submit that the MCR OX-2 antibody (see the attached abstract of a publication, J.Exp.Med. (1992), which discloses that this antibody has specificity for DCs in rats whereas the antibody of the present invention, M-DC8, is specific for human DCs. According to this abstract , the MCR OX-62 antibody detects the alpha-chain of an integrin molecule. Whereas the M-DC8 antibody of the present invention binds to <u>human</u> DCs not rat DCs and binds to a different epitope on the PSGL-1 membrane molecule. Thus, it is requested that this rejection be withdrawn.

Rejections under 35 U.S.C. § 102(e)

Claims 1, 3, 4, 6, 10 and 16-17 are rejected as being clearly anticipated by U.S. Patent No. 5,766,570 ("the '570 patent"). Applicants allege that the '570 patent teaches a monoclonal antibody which reacts with DCs and not other PBMCs, a cell line (hybridoma) that produces the antibody and a method of preparing the antibody. The Examiner acknowledges that the HB15 epitope is the same as the CD83 and is a cell surface marker on mature DCs.

Applicants' claims 1 and 16 are directed to an antibody which binds with an epitope on human DCs displaying one or more surface markers of both immature and mature human DCs. The '570 patent's antibody only binds to the epitope on mature DCs and not immature DCs. In view of these clarifying amendments to the claims and the above arguments, it is requested that this rejection be withdrawn.

CONCLUSION

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date June 15, 2004

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J Exp Med. 1992 Jun 1;175(6):1457-65. Related Articles, Links

The MRC OX-62 antigen: a useful marker in the purification of rat veiled cells with the biochemical properties of an integrin.

Brenan M, Puklavec M.

Medical Research Council Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, England.

A mouse immunoglobulin G1 monoclonal antibody (mAb), MRC OX-62 (OX-62), was raised against density gradient-enriched rat veiled (dendritic) cells obtained from lymph. In suspensions of lymphoid cells, the OX-62 mAb only labeled cells with the characteristics of veiled cells. The OX-62 mAb was used with a magnetic cell sorter to enrich or deplete veiled cells, and the enriched veiled cells were potent stimulators in the primary allogeneic mixed leukocyte reaction. Immunohistochemical staining of tissue sections showed that the OX-62 mAb did not label all classical dendritic cells and was not restricted to this cell type. In lymphoid tissues, the labeling correlated with dendritic cells, but in skin, major histocompatibility complex class II+ cells were OX-62-, while another CD3+ cell with dendritic morphology was strongly OX-62+. It seems that the OX-62 mAb may be restricted to dendritic cells and probably to gamma/delta T cells. The OX-62 mAb will be of use in delineating minor subsets of cells with dendritic morphology in various tissues. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of veiled cell-enriched populations immunoprecipitated with the OX-62 mAb gave bands with the biochemical characteristics of an integrin. The OX-62 mAb recognized the alpha-like subunit.
PMID: 1588275 [PubMed - indexed for MEDLINE]

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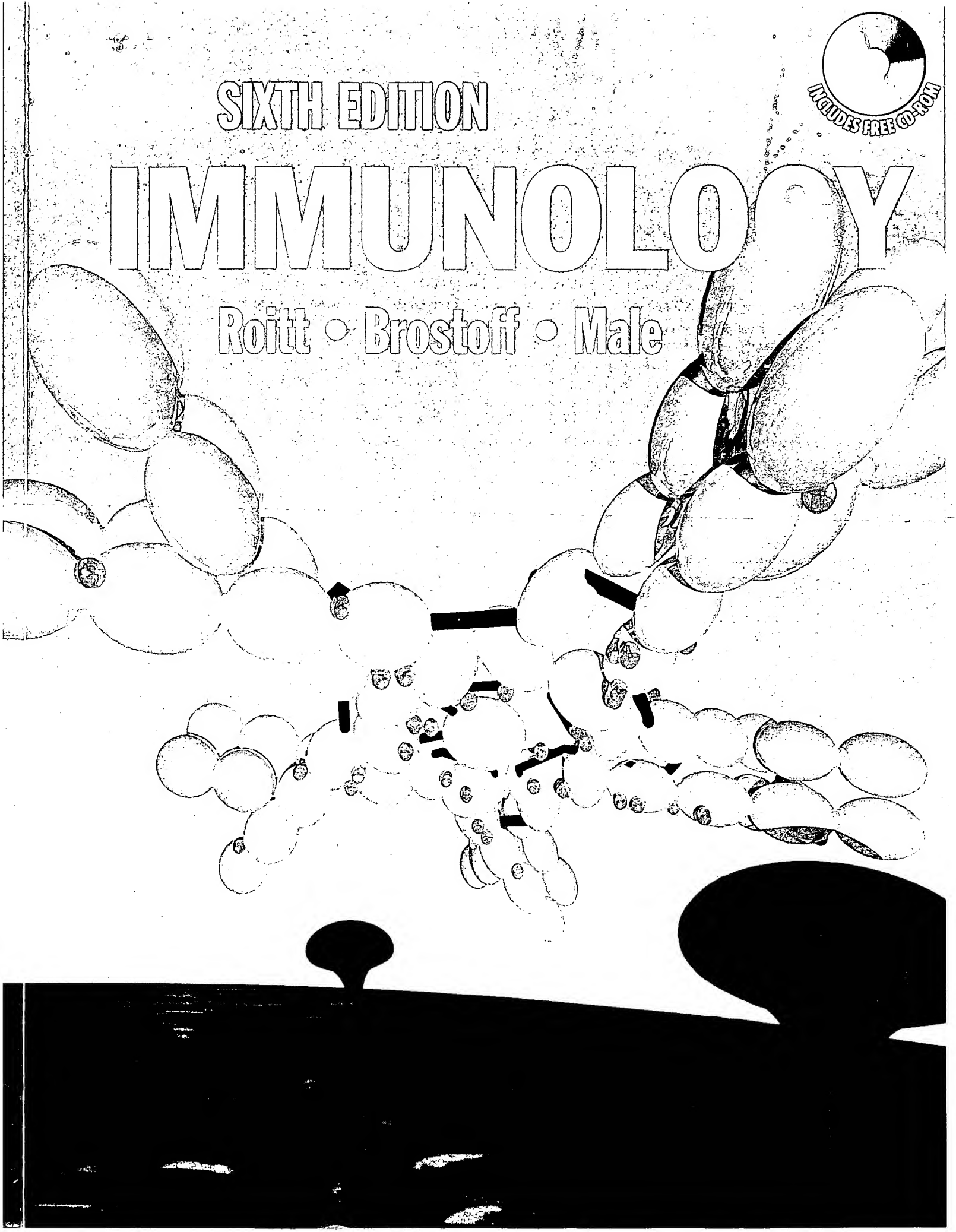

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2 Cells, tissues and organs of the immune system

- **Most cells of the immune system** derive from haemopoietic stem cells.
- **Development and differentiation of different cell lineages** depend on cell interactions and cytokines.
- **Each cell type expresses characteristic surface molecules (markers)** which identify them.
- **Phagocytic cells** are found in the circulation (monocytes and granulocytes) and reside in tissues (e.g. Kupffer cells in the liver).
- **Eosinophils, basophils, mast cells and platelets participate in the inflammatory response.**
- **Antigen-presenting cells** are required by T cells to enable them to respond to antigens.
- **B and T lymphocytes express antigen receptors,** which are required for the antigen recognition.
- **There are two major subpopulations of T lymphocytes** which have helper and cytotoxic activities.
- **B cells** can differentiate into antibody-secreting plasma cells, following activation.
- **Lymphoid organs and tissues** are either primary (central) or secondary (peripheral).
- **Lymphoid stem cells develop and mature within the primary lymphoid organs** – the thymus and bone marrow; this process is called lymphopoiesis.
- **T lymphocytes developing in the thymus are subject to positive and negative selection processes.**
- **The diverse antigen repertoires found in mature animals** are generated during lymphopoiesis, by recombination of gene segments encoding the TCR and Ig.
- **Mammalian B cells develop mainly in the fetal liver and from birth onwards in the bone marrow.** This process continues throughout life. B cells also undergo a selection process at the site of B-cell generation.
- **Lymphocytes** migrate to, and function in, the secondary lymphoid organs and tissues.
- **The systemic lymphoid organs** include the spleen and lymph nodes.
- **The mucosa-associated lymphoid tissue (MALT)** includes all the lymphoid tissues associated with mucosae.
- **Peyer's patches** are a major site of lymphocyte priming to antigens crossing mucosal surfaces of the small intestine.
- **Lymphoid organs** protect different body sites; the spleen responds to blood-borne antigens; the lymph nodes respond to lymph-borne antigens and the MALT protects the mucosal surface.
- **Most lymphocytes recirculate around the body;** there is continuous lymphocyte traffic from the blood stream into lymphoid tissues and back again into the blood via the thoracic duct and right lymphatic duct.

In Chapter 1, we encountered the two major groups of cells, lymphocytes and phagocytes, which comprise the immune system. These and other specialized cells that provide protection against invading organisms are found throughout the body within the blood stream, in specialized organs – the lymph nodes and spleen and beneath the epithelial tissues lining the respiratory, gastrointestinal and genitourinary sytems. These cells derive mainly from undifferentiated 'self-renewing' haemopoietic stem cells (HSCs) through a process of differentiation (*Fig. 2.1*). This is mediated by microenvironmental factors including cell-to-cell interactions and the presence of soluble or membrane-bound cytokines. In addition to their different appearances, cells can be distinguished by their surface markers, which have been collated into the 'CD system', described below and in Appendix 2.

Totipotent HSCs are found in the yolk sac, liver, spleen, bone marrow and in some mesenchymal areas of the embryonic and fetal mammal. After birth and throughout adult life they are normally found only in the bone marrow, where they give rise to four major cell lineages: erythroid (erythrocytes), megakaryocytic (platelets), myeloid (granulocytes and mononuclear phagocytes) and lymphoid (lymphocytes), the latter two being the most important in terms of protection against exogenous pathogens. Antigen-presenting cells are largely, but not exclusively, derived from myeloid precursors. The myeloid and lymphoid lineages are both critical to the functioning of the immune system.

CELLS OF THE INNATE IMMUNE SYSTEM

Phagocytes

Phagocytes belong to two major lineages – monocytes/macrophages and polymorphonuclear granulocytes. The latter have a lobed, irregularly shaped (polymorphic) nucleus. They are classified into neutrophils, basophils and eosinophils, on the basis of how their cytoplasmic granules stain with acidic and basic dyes. The three types of cells have distinct effector functions. Most numerous are the neutrophils, also called PMNs (polymorphonuclear neutrophils), which constitute the majority of leucocytes (white blood cells) in the blood stream (around 60–70% in adults). The other family of phagocytes consists of circulating cells, the monocytes and of cells that reside within the interstitium of various organs (e.g. spleen, liver, lungs) where they display distinctive morphological features and perform diverse functions.

Mononuclear phagocytes are widely distributed throughout the body

The mononuclear phagocyte system has two main functions, which result from the activities of two different types of bone marrow-derived cells:

- 'Professional' phagocytic macrophages, whose main role is to remove particulate antigens.
- Antigen-presenting cells (APCs), whose role is to take up, process and present antigenic peptides to T cells.

antibodies (mAb). A systematic nomenclature has been developed, in which the term CD (Cluster Designation) refers to groups (clusters) of mAb, each cluster binding specifically to a particular molecule. The CD system derives from analysis of mAb against human leucocyte antigens, produced mainly in mice. The work is carried out in many laboratories worldwide, and a series of International Workshops determine the patterns of mAb binding to different leucocyte populations, and the molecular weight of the markers. Monoclonal antibodies with similar characteristics, defined by these criteria, are grouped together and given a CD number. However, it is now customary to use the CD marker to indicate the molecule recognized by each group of monoclonal antibodies (a list of CD markers is given in Appendix 2).

Molecular markers are further defined according to the information they offer about the cell. For example:

- Lineage markers identify a specific lineage, for example CD3, found only on T cells.
- Maturation markers are transiently expressed during differentiation, for example CD1 present on developing thymocytes but not on mature T cells.
- Activation markers, for example the low-affinity T-cell growth factor (IL-2) receptor (CD25) is only expressed when cells are stimulated by antigens or mitogens.

Although it is sometimes useful to define markers in this way, it is not always possible to do so. A maturation marker for one lineage is sometimes an activation marker for the same lineage. For example, CD10 present on immature B cells is lost on mature B cells but reappears on activation. Furthermore, 'activation' markers may already be present at low density on cells, but increase following activation. An example of this is provided by MHC class II molecules that show increased expression on monocytes following their activation by IFNγ.

There are families of cell markers

Cell surface molecules belong to different families which have probably evolved from a few ancestral genes. These families are distinguished by their molecular structure and include the following major groups:

- The *immunoglobulin superfamily* comprises molecules with structural characteristics similar to those of the immunoglobulins. This family includes CD2, CD3, CD4, CD8, CD28, MHC class I and II and many more.
- The *integrin* family consists of heterodimeric molecules with α and β chains. There are several integrin subfamilies; all members of a particular subfamily share a common β chain, but each has a unique α chain. One subfamily (the β_2-integrins) uses CD18 as the β chain. This chain can be associated with CD11a, CD11b or CD11c or αd – these combinations make up the lymphocyte function antigens LFA-1, Mac-1 (CR3), p150,95 and $\alpha d\beta_2$ surface molecules respectively – and are commonly found on leucocytes. A second subfamily (the β_1-integrins) has CD29 as the β chain, again associated with various other peptides and includes the VLA (very late activation) markers.
- *Selectins* (CD62, E, L and P), expressed on leucocytes (L) or activated endothelial cells and platelets (E and P). They have lectin-like specificity for a variety of sugars expressed on heavily glycosylated membrane glycoproteins, for example CD43.
- *Proteoglycans,* typically CD44, have a number of glycosaminoglycans (GAG) binding sites (e.g. for chondroitin sulphate), and bind to extracellular matrix components (typically, hyaluronic acid).

Other families include the tumour necrosis factor (TNF) and nerve growth factor (NGF) receptor superfamily, the C-type lectin superfamily, the family of receptors with seven transmembrane segments (tm7) and the superfamily with four membrane-spanning segments (tm4, e.g. CD20).

It should be emphasized that markers expressed by lymphocytes can often be detected on cells of other lineages, for example CD44 (commonly expressed by epithelial cells). Surface molecules can be demonstrated using fluorescent antibodies as probes (*Fig. 2.29*). This is exploited by the technique of flow cytometry, which can enumerate and separate cells on the basis of their size and fluorescent staining (see *Fig. 27.9*), and which has allowed a detailed dissection of lymphoid cell populations.

The major functions of the above families of marker molecules is to allow the lymphocytes to communicate with their environment. They are extremely important in cell trafficking, adhesion and activation.

T cells

T cells can be distinguished by their different antigen receptors

The definitive T-cell lineage marker is the T-cell antigen receptor (TCR). There are two defined types of TCR: one is a heterodimer of two disulphide-linked polypeptides (α and β); the other is structurally similar but consists of γ and δ polypeptides. Both receptors are associated with a set of five polypeptides, the CD3 complex, and together form the T-cell receptor complex (TCR–CD3 complex; see Chapter 5). Approximately 90–95% of blood T cells are αβ T cells and the remaining 5–10% are γδ T cells.

Immunofluorescent demonstration of T-cell markers

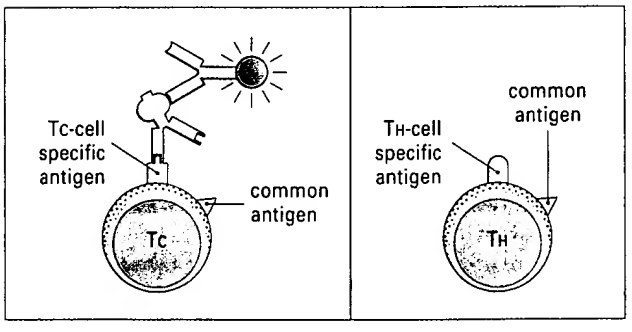

Fig. 2.29 Mouse monoclonal antibodies directed towards a T-cell subset-specific antigen on a T-cytotoxic (Tc) cell, will bind to such cells, but not to T-helper (TH) cells (e.g. CD8). The bound antibody is detected using antibodies to mouse immunoglobulin coupled to a fluorescent molecule. This provides a method for identifying and enumerating T-cell subsets.

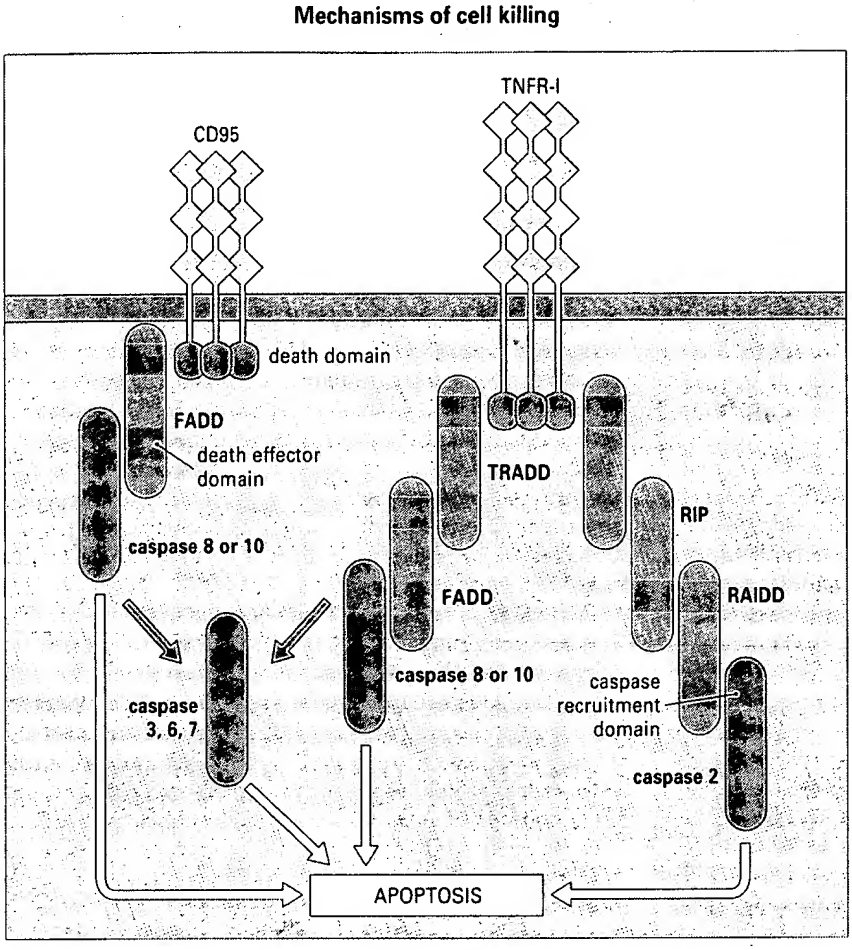

Fig. 10.9 Ligation of CD95 or TNFR-1 causes trimerization of the receptors. Death domains in the cytoplasmic portion of CD95 bind to the adaptor protein FADD (=MORT-1), which recruits caspase 8 or 10. TNFR-1 can activate either caspase 8 or 10, via TRADD and FADD, or caspase 2 via RIP and RAIDD. Caspase 8 can further activate other caspases, and these in concert lead to apoptosis of the target cell.

Activated caspase 8 can cleave and activate other caspases, in addition to its own direct actions in the pathways of apoptosis (*Fig. 10.9*).

Granules of cytotoxic T cells contain perforin and granzymes

It was originally thought that all cytotoxicity was caused by the release of granule proteins onto the target cell. Indeed the processes described above were only identified when it was realized that cells which lack granules could still kill targets. The specific granules of NK cells and Tc cells contain several proteins, including perforin and granzymes (granule-associated enzymes). After binding to its target, the Tc cell directs its granules towards the membrane adjoining the target. Then, in a Ca^{2+}-dependent phase, the granule contents are discharged into the cleft between the two cells. This process can be seen in time-lapse video microscopy (*Fig. 10.10*).

Perforin is a monomeric pore-forming protein that is related both structurally and functionally to the complement component, C9. The vesicles also contain a serine esterase that may be involved in the assembly of the lytic complex. In the presence of Ca^{2+}, the perforin monomers bind to the target cell membrane and polymerize to form transmembrane channels. Although in close contact with the perforin, the Tc cell survives and can continue to kill further targets. It is thought to be protected from autodestruction by a proteoglycan (chondroitin sulphate A) which is also present in the vesicles, and which may bind to and inactivate the perforin. Perforin-knockout mice have Tc cells which display reduced but still functional cytotoxicity, implying that perforin cannot be the only mechanism used by these cells.

Granzymes are a collection of serine esterases (enzymes) which are also released upon granule exocytosis and become active after release. They are not essential for cytotoxicity, as cells lacking granzymes may still be cytotoxic. Some of the granzymes may interact with intracellular pathways in the target cell to activate mechanisms which trigger apoptosis and DNA degradation. In fact, it is notable that granzyme B has the same unusual specificity as the caspases (see above). In order to activate apoptosis pathways in the target cell, the granzymes need to gain access to the cytoplasm. It has been proposed that perforin and granzymes act synergistically; the granzymes enter the target cell via pores created by perforin. The ways in which granule proteins can contribute to cytotoxicity are shown in *Figure 10.11*.

To summarize, $CD8^+$ Tc cells use both FasL and granule release to kill their targets, $CD4^+$ Tc cells use principally FasL, and NK cells use primarily their granules. TNF may contribute to the cytotoxic damage produced by any of these cells.

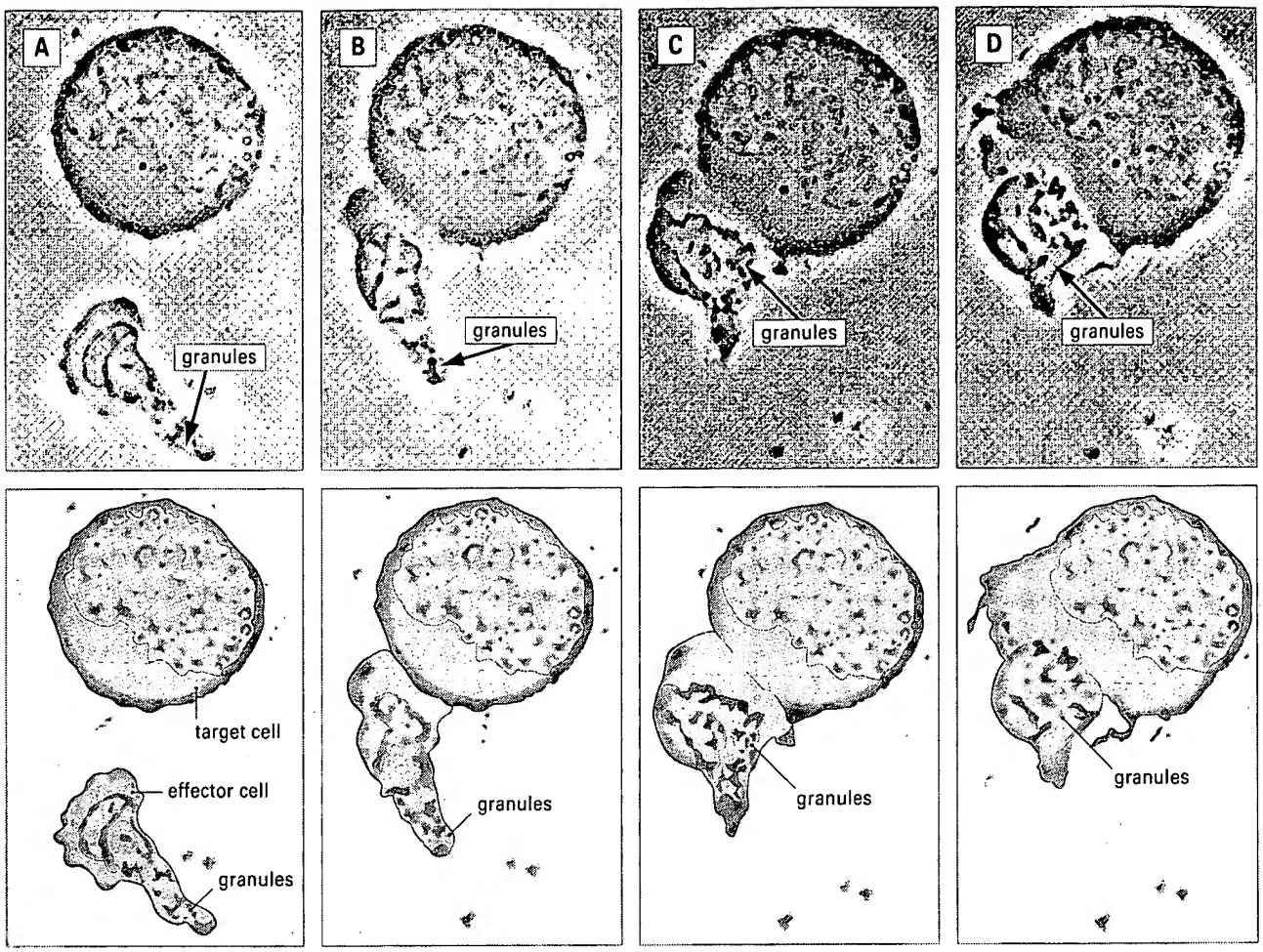

Fig. 10.10 Intracellular reorganizations during effector–target cell interaction. Early events in the interaction of Tc with specific targets were studied with high-resolution cinematographic techniques. Four frames (together with interpretative drawings) are shown, taken at different times, of a Tc interacting with its target. The location of the granules within the effector cell is indicated in each case. Before contact with the target (a), the effector had granules located in a uropod at the rear, and was seen to move randomly by extending pseudopods from the organelle-free, broad leading edge of the cell. Within 2 minutes of contacting the target (b), the Tc had begun to round up and initiate granule reorientation (c). After 10 minutes (d), the granules occupied a position in the zone of contact with the target, where they appear to be in the process of emptying their contents into the i ntercellular space between the two cells. (Courtesy of Dr V.H. Engelhard.)